REMARKS

Claim Objections

Applicants have corrected Claim 9 according to the suggestions in the Office Action by deleting the phrase "a protein" and replacing it with "proteins" and Claim 10 by properly labeling the list of sequestering materials with the letters (a), (b) and (c).

Claim Rejections – 35 USC § 112

The Office Action dated June 4, 2002, had rejected Claims 48-50 under §112, first paragraph, as containing subject matter not described in the specification in such a way as to enable one skilled in the art to which it pertains, or with which it is most nearly connected, to make and/or use the invention. Applicants concur with the statement that "every mRNA of a cell is not necessarily one involved in cell growth and differentiation," Office Action dated 6/3/02, page 3, and have amended claim 48 to recite a "preselected target 3' polyadenylated messenger RNA sequence known to be involved in the modulation of cell growth or differentiation," in order to clarify that the invention, as encompassed by claims 48-50, contemplates the preselection and exogenous addition of an mRNA for a protein already known to be involved in cell growth and differentiation. *See* Specification, page 4, line 27. The claimed method is designed to work on exogenous mRNA to allow ease of experimental manipulation. *See* Specification, page 4, lines 21-22. Adding a preselected mRNA known to be involved in cell growth and differentiation allows one to conclude that if an agent modifies the stability of such an mRNA, then it is necessarily one involved in cell growth and differentiation. Hence, the essence of the claimed invention is not to work with "random or unknown sources of mRNA," (Office Action page 3) but rather to work with mRNAs known to be

involved in cell growth or differentiation. Such mRNAs involved in cell growth or differentiation are readily known by one skilled in the art, and the genes encoding them have been sequenced, so that isolating or synthesizing such mRNAs does not require undue experimentation. One having ordinary skill in the art is able to obtain any mRNA of a known growth or differentiation factor without undue experimentation, as for example by the well known method of Melton et al., Nucleic Acids Res. 12:7035-7056 (1984) or by the use of commercially available kits such as the Promega Riboprobe In Vitro Transcription Systems. In addition, the specification provides an example of RNA production at page 52, lines 20-27. Hence, a step of first isolating or synthesizing an mRNA for a protein known to be involved in cell growth and differentiation is not necessary in the system of the present invention. Applicants therefore respectfully request withdrawal of the rejection.

Claims 21, 23-33, 35-45, 51-52, and 55-56 have been rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

Claim 21 had been rejected because it recited "said turnover" in step (d), and because there was no antecedent basis for this term in the claims. The Office Action correctly noted that applicants' response of 3/13/2002 continued to recite "said turnover" in step (d), even though the other steps had been amended to recite "deadenylation and degradation." Applicants apologize for this oversight and have amended claim 21, deleting "said turnover" in step (d) and replacing it with "deadenylation and degradation." Similarly, applicants have amended claim 27 which depends from claim 21, deleting "said turnover" and replacing it with "deadenylation and degradation." Because claims 21 and 27 have been amended to recite the correct terms, applicants respectfully request withdrawal of the rejection.

Claims 23 and 35 have been rejected as indefinite because they were directed to a method but depend from claim 1 which recites a system. According to the Office Action, it was unclear whether claims 23 and 35 were intended to recite a system or a method. Applicants have amended claim 23, by deleting "The method of claim 1" and replacing it with "The method of claim 21," and claim 35, by deleting "The method of claim 1" and replacing it with "The method of claim 33." Because these corrections make it clear that claims 23 and 35 are intended to recite a method, thereby rendering claims 23 and 35 definite, applicants respectfully request that the rejection be withdrawn.

Claim 33 has been rejected as indefinite in the recitation of "turnover" in steps (d) and (e). The Office Action correctly noted that "claim 33 was amended in the response of 2/11/00 to recite 'deadenylation and degradation' in lines 7 and 8 (steps (d) and (e)); however, both the 'clean' and marked-up copies of the claim recite 'turnover' in the amendment filed 3/13/02." Final Office Action, page 4. Applicants sincerely apologize for the inadvertent reversion to the previous version of claim 33 and have amended claim 33 to recite "deadenylation and degradation" in steps (d) and (e) in place of "turnover" and "said turnover," respectively. Because claim 33 is now definite, applicants respectfully request that the rejection be withdrawn.

Claim 38 has been rejected as indefinite because it recites "deadenylation and degradation" of target mRNA in the method of claim 36, where claim 36 depends from claim 33. Neither of claims 33 or 36 recite "deadenylation and degradation." *See* Final Office Action, page 5. Since claim 33 has been amended (*supra*) to recite "deadenylation and degradation," claim 38 is no longer indefinite for being unclear as to what is meant by "deadenylation and degradation," and applicants respectfully request withdrawal of the rejection.

Claim 51 has been rejected as indefinite. *See* Final Office Action, page 5. Applicants have amended the preamble of claim 51, deleting "A method determining whether an endogenous molecule participates in the deadenylation or degradation of RNA or regulation thereof comprising" and replacing it with "A method of determining whether an endogenous molecule modulates the deadenylation or degradation of a target RNA sequence comprising." Additionally, applicants have amended part (c), deleting "said regulation" and replacing it with "deadenylation and degradation." Such amended language is consistent with the language recited in independent claim 47, which is allowed by the Final Office Action, and eliminates any confusion associated with the words "participates" and "regulation." Accordingly, applicants respectfully request withdrawal of the rejection.

Claim 52 has been rejected as indefinite for depending from claim 53. Applicants respectfully submit that claim 52 depends from claim 51 rather than from claim 53 as stated by the Office Action. *See* Final Office Action, page 5. In view of applicants' amendments to claim 51, claim 52 is no longer indefinite. Applicants therefore respectfully request withdrawal of the rejection.

Claim 55 has been rejected as indefinite. Final Office Action, pages 5-6. Applicants have amended the claim to alleviate confusion surrounding the variety of items found in step (a), and therefore respectfully request that the rejection be withdrawn.

Claim 56 has been rejected as indefinite for reciting that the endogenous molecule in the method of claim 51 "is isolated." *See* Office Action, page 6. Applicants have amended claim 56, deleting "is isolated" and replacing it with "is an isolated molecule" as suggested by the Office Action. Additionally, Applicants, in order to be consistent with the amendments to claim 51, have

amended "deadenylation and degradation" to replace "regulation." In view of the foregoing amendments, applicants respectfully request that the rejection be withdrawn.

Claim rejections – Double Patenting

Claims 23 and 35 have been objected to under 37 CFR 1.75 as being substantial duplicates of claim 17. Applicants have amended claim 23 to depend from claim 21 and claim 35 to depend from claim 33, thereby obviating the double patenting rejection. Accordingly, applicants respectfully request that the rejection be withdrawn.

CONCLUSION

It is respectfully submitted that all pending claims have been placed in allowable form.

No fee is believed to be due with this amendment. However, if Applicants are in error, the Commissioner is authorized to charge required fees to Perkins Coie's Deposit Account No. 50-0665.

Respectfully submitted,
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MARKED UP VERSION TO SHOW ALL CHANGES
PURSUANT TO 37 CFR 1.121(b)(1)(iii)

IN THE SPECIFICATION:

On page 2, lines 22 to 28, please delete the paragraph and replace it with the following.

Numerous proteins have been described that interact with some specificity with an ARE, but their exact role in the process of mRNA turnover remains to be defined. For example, proteins which bind to the ARE described above include HuR and other ELAv family proteins, such as HuR (also called HuA), Hel-N1 (also called HuB), HuC and HuD; AUF 1 (four isoforms); [tristetrapolin] tristetraprolin; AUH; TIA; TIAR; glyceraldehyde-3-phosphate; hnRNP C; hnRNP Al; AU-A; and AU-B. Many others have not been extensively characterized.

On page 7, line 4 to 11, please delete the paragraph and replace it with the following.

The method of the present invention is useful for identifying agents which can either increase or decrease the stability of said target RNA sequence. Such agents may be capable of modulating the activity of an RNA binding molecule such as, but not limited to, C-rich element binding proteins and AU rich element binding proteins, examples of the latter including HuR and other ELAv family proteins, such as HuR , Hel-N1, HuC and HuD; AUl 1; [tristetrapolin] tristetraprolin; AUH; TIA; TIAR; glyceraldehyde-3-phosphate; hnRNP C; hnRNP Al; AU-A; and AU-B. This list is provided as illustrative of the types of molecules that may be evaluated in the present invention, but is by no means limiting.

On page 8, line 1 to 13, please delete the paragraph and replace it with the following.

The non-limiting selection of the components of this method are as described above. The aforementioned method is useful, for example, when the RNA stability modifier decreases the stability of said target RNA sequence, and the agent to be identified increases the stability of the target RNA sequence that is decreased by the RNA stability modifier. In addition, the method is useful when the RNA stability modifier increases the stability of the target RNA sequence, and the agent to be identified decreases the stability of the target RNA sequence that is increased by the RNA stability modifier. Non-limiting examples of RNA stability modifiers include C-rich element binding proteins, and AU rich element binding proteins, examples of AU rich element binding proteins, including HuR and other ELAv family proteins, such as HuR, Hel-N1, HuC and HuD; AUF1; [tristetrapolin] tristetraprolin; AUH; TIA; TIAR; glyceraldehyde-3-phosphate; hnRNP C; hnRNP A1; AU-A; and AU-B. This list is provided as illustrative of the types of molecules that may be evaluated in the present invention, but is by no means limiting.

Starting on page 20, line 8 and ending on page 21, line 4, please delete the paragraph and replace it with the following.

The key to the development of the system and methods utilizing the system are based on the discovery that polyadenylate competitor RNA is capable of sequestering proteins that bind polyadenylate and consequently activating the deadenylase enzyme, inducing RNA turnover. As it was heretofore considered that such proteins that bind polyadenylate may contribute to RNA deadenylation, the present finding that such proteins are, in contrast, stabilizers of RNA, led to the realization that such proteins are interacting with and inactivating destabilizing mediators in

vivo. Thus, the present invention is directed to an *in vitro* system capable of recapitulating regulated RNA turnover of an exogenously added preselected target RNA sequence comprising a cell extract depleted of activity of proteins that bind polyadenylate, and a preselected target RNA sequence. In one particular embodiment, the regulated RNA turnover is that modulated by AU-rich element (ARE) regulated RNA turnover. Examples of mRNAs with AU-rich elements include those of, by way of non-limiting example, c-fos; c jun; c-myc TNF-α, GMCSF, IL1-15, and IFN-β. As noted above, AU-rich elements are sites for binding of numerous proteins, including the ELAV family of ARE-binding proteins, such as HuR, He1-N1, HuC and HuD; others include AUF1; [tristetrapolin] tristetraprolin; AUH; TIA; TIAR; glyceraldehyde-3-phosphate; hnRNP C; hnRNP A1; AU-A; and AU-B. In another embodiment, the regulated RNA turnover is that modulated by C-rich element (CRE) regulated RNA turnover, such elements as found in the mRNA of globin mRNAs, collagen, lipoxygenase, and tyrosine hydroxylase. Another mRNA with an as yet uncharacterized sequence element is that of VEGF. The invention, however, is not so limiting as to the particular elements or binding proteins to these elements involved in the regulation of RNA turnover.

IN THE CLAIMS:

Please amend the claims as follows:

4. (Three Times Amended) The system of claim [3] 1 wherein said cytoplasmic extract is obtained from a cell line selected from the group consisting of HeLa cells and a T cell line.

9. (Twice Amended) The system of claim 1 wherein said cytoplasmic extract is selected from the group consisting of:

(a) a cytoplasmic extract which contains polyadenylate competitor RNA;

(b) a cytoplasmic extract which contains a material that sequesters proteins that bind polyadenylate;

(c) a cytoplasmic extract which contains a proteinase that inactivates [a] proteins that bind to polyadenylate; and

(d) a cytoplasmic extract which contains an agent that prevents the interaction between polyadenylate and an exogenous macromolecule that binds to ploydenylate.

10. (Twice Amended) The system of claim 9 wherein the material that sequesters proteins that bind polyadenylate is selected from the group consisting of:

[1.] (a) antibodies to proteins that bind polyadenylate;

[2.] (b) polyadenylate[,]; and

[3.] (c) a combination of antibodies to proteins that bind polyadenylate, and polyadenylate.

15. (Three Times Amended) The system of claim 14 wherein said labeled target 3' polyadenylated messenger RNA sequence is labeled with a moiety [is] selected from the group

consisting of a fluorescent moiety, a visible moiety, a radioactive moiety, a ligand, and a combination of fluorescent and quenching moieties.

21. (Twice Amended) A method for identifying an agent capable of modulating the stability of a target 3' polyadenylated messenger RNA sequence comprising

(a) providing the system of claim 1;

(b) introducing said agent into said system;

(c) determining the extent of deadenylation and degradation of said target 3' polyadenylated messenger RNA sequence; and

(d) identifying an agent able to modulate the extent of [said turnover] <u>deadenylation and degradation</u> as capable of modulating the stability of said target 3' polyadenylated messenger RNA sequence.

23. (Twice Amended) The method of claim [1] <u>21</u> wherein said source of ATP is exogenous.

26. (Amended) The method of claim 25 wherein said labeled target 3' polyadenylated messenger RNA sequence is labeled with a moiety [is] selected from the group consisting of a fluorescent moiety, a visible moiety, a radioactive moiety, a ligand, and a combination of fluorescent and quenching moieties.

27. (Twice Amended) The method for claim 21 wherein said monitoring the extent of [turnover] <u>deadenylation and degradation</u> of said target 3' polyadenylated messenger RNA sequence comprises determining the extent of degradation of said labeled target 3' polyadenylated messenger RNA.

33. (Twice Amended) A method for identifying an agent capable of modulating the stability of a target 3' polyadenylated messenger RNA sequence in the presence of an exogenously added RNA stability modifier comprising

(a) providing the system of claim 1;

(b) introducing said RNA stability modifier into said system;

(c) introducing said agent into said system;

(d) determining the extent of [turnover] deadenylation and degradation of said 3' polyadenylated messenger RNA sequence; and

(e) identifying an agent able to modulate the extent of [said turnover] deadenylation and degradation as capable of modulating the stability of said target 3' polyadenylated messenger RNA sequence in the presence of said exogenously added RNA stability modifier.

35. (Twice Amended) The method of claim [1] 33 wherein said source of ATP is exogenous.

48. (Three Times Amended) A method for identifying an agent capable of modulating cell growth or cell differentiation in a mammal comprising determining the ability of said agent to modulate the stability of a preselected target 3' polyadenylated messenger RNA sequence known to be involved in the modulation of cell growth or differentiation in accordance with claim 19.

51. (Twice Amended) A method of determining whether an endogenous molecule [participates] modulates [in the] deadenylation or degradation of a target RNA sequence [or regulation thereof] comprising

(a) providing the system of claim 1 containing target 3'polyadenylated messenger RNA;

(b) introducing said endogenous molecule into said system; and

(c) monitoring the stability of said target 3' polyadenylated messenger RNA sequence in said system thereby determining whether said endogenous molecule is capable of modulating [said regulation] deadenylation and degradation.

55. (Twice Amended) A method for determining whether an agent is capable of modulating the degradation of a target 3' polyadenylated messenger RNA sequence in the absence of deadenylation comprising

(a) providing a cytoplasmic extract supernatant from a 100,000 x g, 1 hour centrifugation isolated from eukaryotic cells or tissues, said extract depleted of activity of proteins that bind polyadenylate[,]; a source of ATP; and an exogenous target 3' polyadenylated messenger RNA sequence; [in the presence of a nucleotide triphosphate;]

(b) introducing said agent into said cytoplasmic extract; and

(c) monitoring the degradation of said target 3' polyadenylated messenger RNA sequence in said extract thereby determining whether said agent is capable of modulating said degradation.

56. (Amended) The method of claim 51 wherein the endogenous molecule capable of modulating [regulation] deadenylation and degradation is an isolated molecule.